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Zoological Research

Cytogenetic Effects of Pulsing Electromagnetic Field on Domestic Pig Lymphocytes *in Vitro*

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Abstract: The effects of pulsing electromagnetic fields (PEMFs) on cells are very important subjects in the field of bioelectromagnetics. In this experiment, the cytogenetic effects of PEMF on domestic pig lymphocytes were tested *in vitro*. Pig lymphocytes in RPMI 1640 medium were exposed to PEMFs of 100 kHz and 200 kHz for 12, 24 and 48 hours. Chromosomal aberrations (aneuploidy, breaks, gaps, *et al.*) were significantly increased in exposed cultures, and of these aberrations, 56% chromosomal or chromatid breaks and 42% gaps induced by PEMFs were the points of pig chromosomal fragile sites. The baseline frequency of sister-chromatid exchange (SCE) increased after exposing lymphocytes continuously to PEMFs of 100 kHz and 200 kHz for 48 hours. These results suggested that the exposure to PEMFs might induce a type of DNA lesion and chromosomal aberrations.

Key words: Pulsing electromagnetic fields; Peripheral blood lymphocyte; Chromosomal aberrations; Fragile sites

The pulsing electromagnetic fields (PEMFs) resulting from lightning strokes, nuclear bursts and radar transmitters as well as from high-voltage electrical energy generation are fairly well known. Electromagnetic fields have been shown to influence a variety of biological processes, including cell proliferation (Cossarizza *et al.*, 1989), DNA synthesis (Sollazzo *et al.*, 1996) and DNA transcription (Lin *et al.*, 1996), and have been clinically applied to stimulate bone healing (Fini *et al.*, 1995). Physical and chemical agents that interfere with DNA synthesis are known to influence the formation of sister-chromatid exchange (SCE) and chromosomal aberration (CA). So these two events are being extensively used as sensitive indicators of genetic damages in both laboratory animals and human. Laboratory investigations with controlled electromagnetic exposure conditions have given conflicting cytogenetic effects. Although positive CA was reported in *Ehrlich ascites* tumor cells in mice (Mitchell *et al.*, 1978), as well as in bovine and human lymphocytes (Ambrosio *et al.*, 1985; Khalil & Qassem, 1991), negative findings about CA have been shown for human lymphocytes (Cohen *et al.*, 1986). Negative results regarding the level of SCE have also been reported in Chinese hamster cells (Takabashi *et al.*, 1987) and in bovine (Ambrosio *et al.*, 1985) and human lymphocytes (Khalil & Qassem, 1993). Similar discrepancy is encountered in long-term studies. Strong evidence for potential genetic hazards due to electromagnetic fields has been provided by one study but not by another one.

In view of these conflicting results, we further evaluated the possible cytogenetic effects of PEMFs on domestic pig lymphocytes *in vitro* with respect to CA and SCE, which have been suggested to be benchmarks for implementation of environmental control and medical surveillance.

1 Materials and Methods

1.1 Cell culture and exposure

Domestic pig (Landrace) lymphocytes from peripheral blood (jugular vein) were cultured for 72 hours at 38.2℃ in RPMI 1640 medium supplemented with 20% heatinactivated fetal bovine serum, 3%

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phytohemagglutinin (PHA) and double antibiotics. After culture initiation, culture flasks were placed in a BTEM Cell (Zou *et al*., 1999), a source of pulsing electromagnetic fields, and were kept in the electromagnetic fields for 12, 24 and 48 hours respectively. After the specified exposure time, the cultures were transferred, for the remaining culture period, to an equivalent incubator along with the control cultures where no electromagnetic field was detectable. The parameters of PEMFs sources were: rising time of pulse 1.2 ns, duration time 2.4 ns, amplitude 80 - 100 V, repetition 100 kHz and 200 kHz. To each culture 0.04 μg/mL colcemid was added 3 hours before harvested to prepare chromosome.

1.2 Chromosome preparation and G-band display

After harvesting the cultures, chromosome preparations were made according to a standard protocol and the slides were air-dried and stained using Giemsa technique (Chen *et al*., 1993). Then the types of chromosomal aberrations were analyzed among 300 well-spread metaphases in each group. To locate the chromosomal breaks and gaps, the G-banded karyotypes were established after having determined the chromosomal aberrations. The G-band of chromosome was displayed according to Chen *et al*. (1993).

1.3 Analysis of sister chromatid exchange

The method of pig lymphocyte culture and exposure to PEMFs was the same above. In the flasks containing cells for sister-chromatid exchange (SCE) studies, bromodeoxyuridine (BrdU. final concentration 10 μg/mL) was added 48 hours before cell collection. Chromosome preparations were made as above. The frequency of induced SCE was determined by scoring 100 metaphases for each culture.

T-test was adopted to determine whether there is any significant difference between the experiment groups and the controls.

2 Results

2.1 Influence of PEMFs on the frequency of chromosomal aberrations

The results of the frequency of chromosomal aberrations are listed in Table 1. It showed: ①the longer the time exposed, the more percentages of chromosomal aberrations were obtained; ②among the chromosomal aberrations analyzed in exposed groups, chromosomal and chromatid breaks were the most frequent, then the gaps (Plate Ⅰ: 1 - 4).

2.2 The relation between the chromosomal breaks, gaps induced by PEMFs and the fragile sites

After exposed by PEMFs for different intervals, both of chromosomal breaks and gaps increased (Table 1). The relation between the chromosomal breaks, gaps induced by PEMFs and fragile sites was summarized in Table 2, and the localizations of some breaks and gaps could be seen from the chromosomal G-binding photographs (Plate Ⅰ: 5 - 9). Of the induced breaks (totally 293) and gaps (totally 71), about 56% of the breaks and 42% of the gaps took place at the points of chromosomal common fragile sites (Table 2).

2.3 Influence of PEMFs on the frequency of SCE

The frequency of SCE was determined by analyzing 100 metaphases for each culture. With respect to the SCE levels per cell, as can be seen in Table 3, no significant variability was observed between the cells exposed to PEMFs of 100 kHz and 200 kHz for 12 or 24 hours and the controls. However, the increase in the rate of the exchanges was significant ($P < 0.05$)

Table 1 The frequency of chromosomal aberrations of domestic pig lymphocytes exposed *in vitro* to PEMFs for different periods

Exposure time (h)	100 kHz								200 kHz							
	A	B	D	R	Q	T	G	Total/%	A	B	D	R	Q	T	G	Total/%
0	1	23	0	1	2	0	3	10	0	19	0	0	2	0	5	8.7
12	2	38	1	0	7	8	7	21*	2	36	1	1	3	0	9	17.3*
24	3	43	1	1	4	8	10	23.3*	4	47	2	0	7	5	12	25.7*
48	3	60	1	2	8	12	15	33.7*	3	69	2	1	8	9	18	36.7*

A: aneuploidy; B: breaks; D: dicentrics; R: rings; Q: quadriradials; T: triradials; G: gaps.

* means significant difference between the control and exposure group ($P < 0.05$).

Table 2 The distribution of chromosomal breaks, gaps induced by PEMFs

Fragile sites	Number of breaks	Number of gaps	Fragile sites	Number of breaks	Number of gaps
1p22	1	0	6q32	8	2
1p14	6	1	7q21	11	3
1q11	10	2	7q23	7	1
1q26	9	1	8q12	11	1
2p14	6	0	9q21	4	1
2q12	2	0	11q12	8	2
3p14	7	2	12q11	13	3
3q14	8	3	13q34	6	0
4p14	4	0	13q46	5	1
6p12	7	1	14q26	8	0
6q28	3	2	15q14	3	1
6q31	7	1	18q21	10	2
Total	70	13	Total	94	17

Table 3 The frequency of SCE induced by PEMFs

Exposure time (h)	100 kHz SCE per metaphase	200 kHz SCE per metaphase
0	10.1 ± 2.2	11.4 ± 2.1
12	9.8 ± 1.9	10.5 ± 2.6
24	11.5 ± 1.4	12.1 ± 2.2
48	14.5 ± 1.9*	15.5 ± 1.8*

* means significant difference relative to the control group ($P < 0.05$); SCE is represented by mean ± *SD*.

when cells were continuously exposed to PEMFs of 100 kHz and 200 kHz for 48 hours.

3 Discussion

With the development of industry and communication, more and more electromagnetic fields are existing in occupational and residential environment. It is necessary to evaluate the potential harm of electromagnetic fields. We have found that PEMFs with different parameters can disturb many biological processes including the effects on human lymphocyte immunity and on the low permeability-resisting of human red blood cell membrane (Zou *et al*., 1999). The results of the present studies indicated that the exposure of PEMFs of 100 kHz and 200 kHz could lead to cytogenetic effects on peripheral pig lymphocytes *in vitro*. These results are in consistence with previous reports having discovered that *in vivo* and *in vitro* exposures of animal or human cells to electromagnetic fields may have cytogenetic effects (Ambrosio *et al*., 1985; Khalil & Qassem, 1991; 1993).

We also found that many breaks and gaps induced by PEMFs in the pig genome took place at some specific sites on chromosomes. Those points are fragile sites. Fragile sites on chromosomes are non-randomly distributed points at which the chromosomes are liable to break under the influence of some factors. Often these factors are some chemical agents, including thymidine starvation, mutagens, carcinogens and clastogens (Yunis *et al*., 1987). However, we discovered that PEMFs could also cause chromosomes to break at the fragile sites. This is the first time to report that PEMFs could induce the expression of fragile sites. Although many results had been reported about the chromosomal aberrations induced by PEMFs, it was not sure why PEMFs could cause such effect. Most of these breaks and gaps induced by PEMFs were located at the fragile sites from our results (Table 2). This suggested that the fragile sites might be the structural basis of cytogenetic effects induced by PEMFs, despite some other forms of chromosome aberrations were observed in our studies. It means that fragile sites are sensitive to the exposure of PEMFs.

It is known that the exposure to electromagnetic fields does not affect the baseline SCE frequency in cultured bovine lymphocytes (Ambrosio *et al*., 1985), Chinese hamster V79 cells (Takahashi *et al*., 1987) and lymphocytes from human (Khalil & Qassem, 1993). Although our results for the two shorter exposure periods (12 and 24 hours) were consistent with these findings, the significant increase in SCE level was observed in the continuously exposed groups for 48 hours. This is consistent with the report of Khalil & Qassem (1991), in which the SCE of human lymphocytes increased after continuously exposed to PEMF for 72 hours. Because the parameters of our PEMFs were not the same as Khalil's, it is hard to determine which parameter will have more effects than the others. The intensity of magnetic field used by Khalil was much bigger than that of ours (our intensity of magnetic field was near zero), and on the other hand, his frequency of magnetic field was lower. However, to produce the significant difference of SCE, our exposure time was only 48 hours. According to previous results (Zou *et al*., 2000), perhaps the intensity of PEMFs was not the key factor to determine what biological effects would be pro-

duced, and maybe the PEMFs in this experiment played a strong role than the low frequent PEMFs did.

Because chromosome damage is always a sensitive indicator for the influences of environmental physical or chemical factors, our results demonstrated that electromagnetic fields could damage chromosome and DNA of domestic pig lymphocytes *in vitro*.

Explanation

1 – 4 are the photographs of pig chromosomes stained with Giemsa and are showing the breaks or gaps induced by PEMFs.

1. 1a is showing the gap of No.1 chromosome; 1b is showing the chromatid break of No.7 chromosome.
2. Showing chromatid break.
3. 3a is showing the break of No.13 chromosome; 3b is showing the gap of No.1 chromosome.
4. 4a is showing the break of No.6 chromosome; 4b is showing the chromatid break.

5 – 9 are the G-banding chromosomes showing the localizations of breaks or gaps induced by PEMFs.

5. Showing the gap took place at 16q21
6. 6a is showing the gap took place at 13q31 and 6b is showing the gap took place at 1p14
7. showing the gap took place at 6q32.
8. showing the gap took place at 6q28.
9. showing the break took place at 4p14.

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脉冲电磁场对家猪淋巴细胞的细胞遗传学效应

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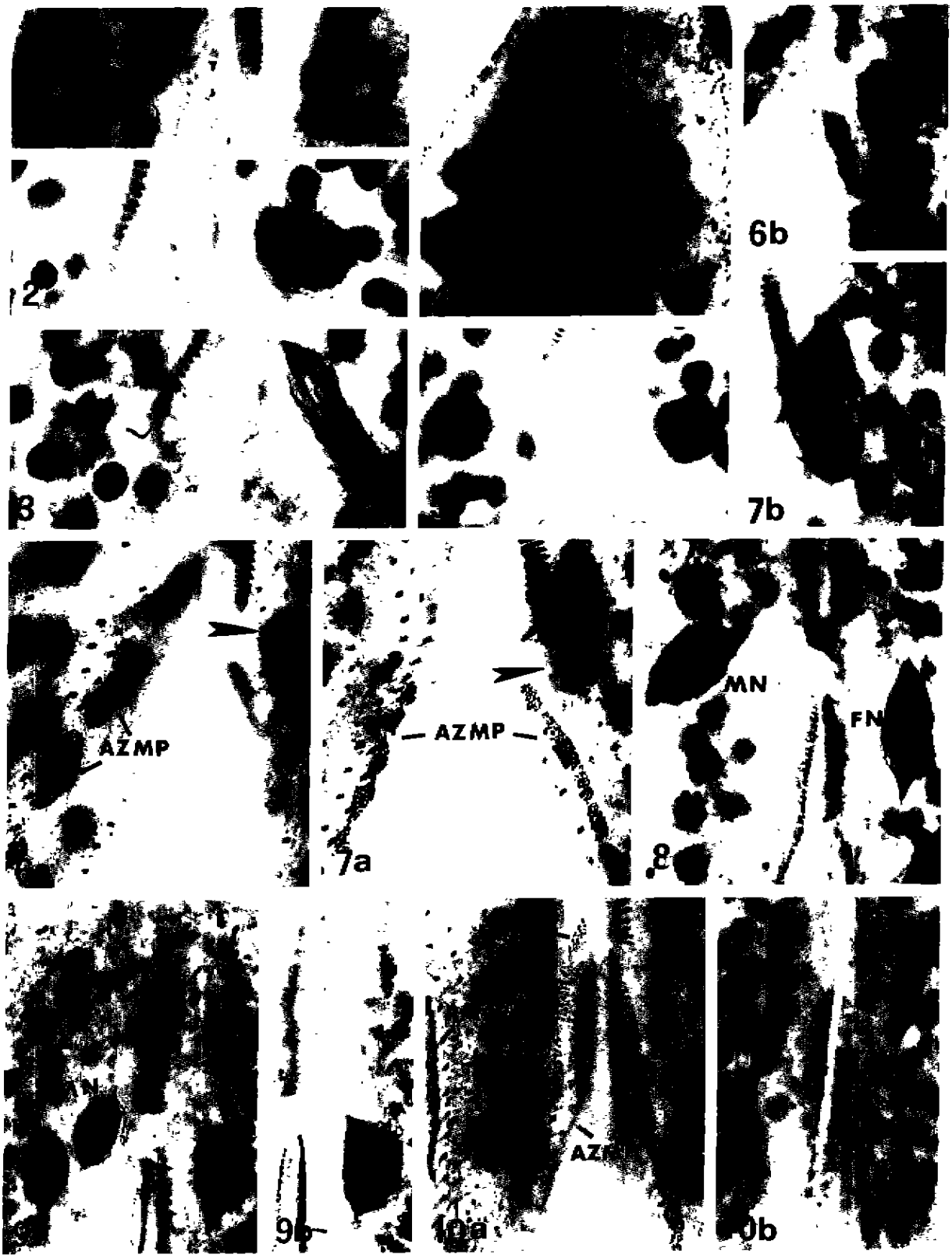
摘要：以家猪外周血淋巴细胞为材料，研究了脉冲电磁场（pulsing electromagnetic fields，简称PEMFs）对细胞的遗传学效应。实验发现，100和200 kHz的PEMFs对家猪的淋巴细胞照射培养12、24、48 h后，染色体畸变（包括非整倍体、染色体断裂等）频率明显高于对照组（$P < 0.05$）。其中，56%的染色体或染色单体断裂和42%的间隙发生在家猪常见染色体脆性位点部位。同时，经100 kHz和200 kHz的PEMFs照射48 h后，淋巴细胞姐妹染色单体交换（SCE）频率也明显高于对照组（$P < 0.05$）。实验结果表明，PEMFs能诱导DNA损伤和染色体畸变。

关键词：脉冲电磁场；外周血淋巴细胞；染色体畸变；脆性位点

中图分类号：Q343，S828 **文献标识码**：A **文章编号**：0254 – 5853(2001)02 – 0089 – 04



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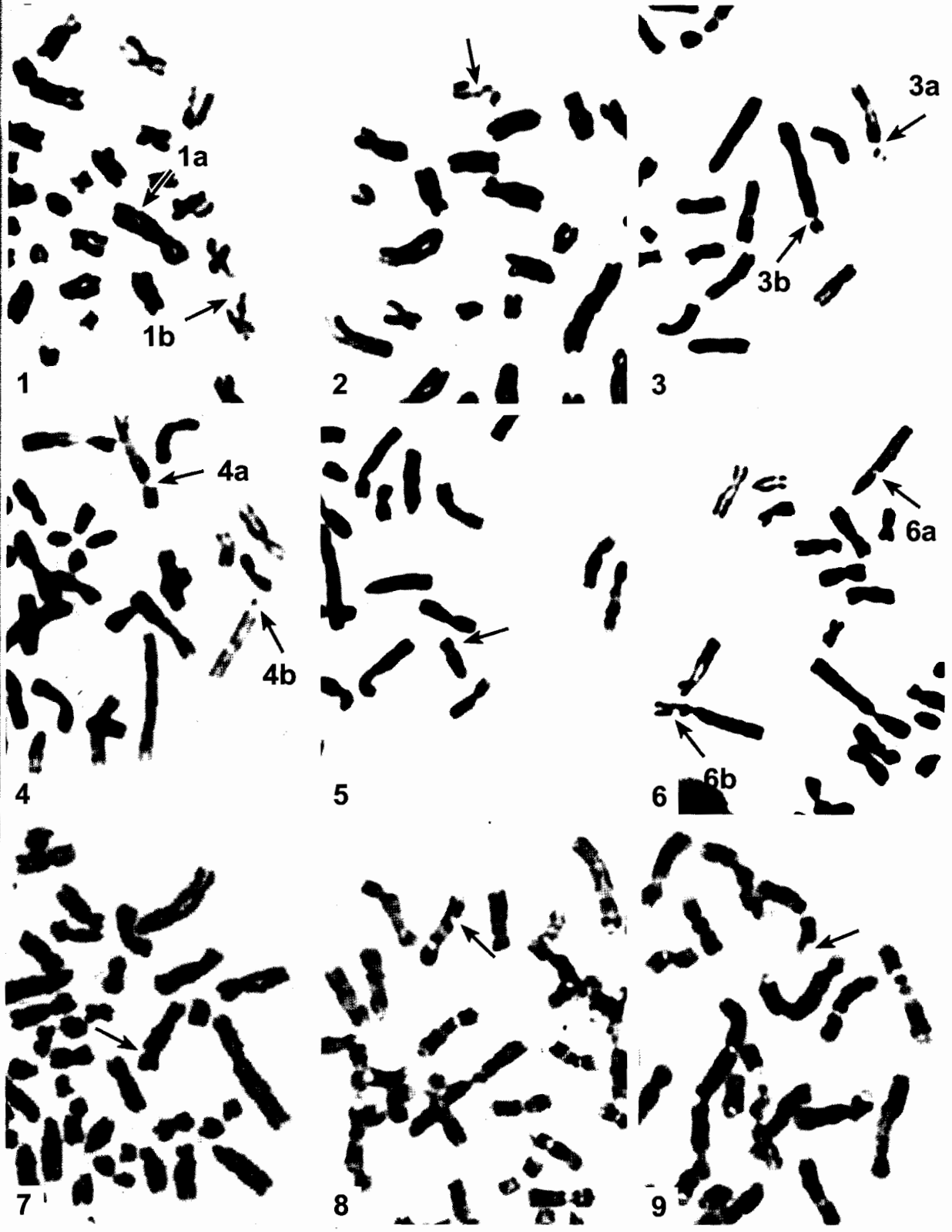

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